Complexity of nutrient enrichment on subarctic peatland soil CO₂ and CH₄ production under increasing wildfire and permafrost thaw

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Abstract. The adverse impacts of excessive soil nutrients on water quality and carbon sequestration have been recognized in tropical and temperate regions, with already widespread industrial farming and urbanization, but rarely in subarctic regions. However, recent studies have shown significant increases in porewater nitrogen (N) and phosphorus (P) concentrations in burned subarctic peatlands and downstream waters, which is a growing concern as climate change leads to increasing wildfires, permafrost thaws, and waterlogged peatlands. In this study, we present the results of a short-term incubation experiment conducted on soils from subarctic bogs and fens, aimed at evaluating the effects of high levels of nutrients on carbon gas production rates. We divided aliquots of the peatland soil samples into separate containers and added artificial porewater to each, enriching them with dissolved inorganic nitrogen (N), phosphorus (P), both, or none for controls. Overall, the fen samples showed higher carbon dioxide (CO₂) and methane (CH₄) production rates at 1, 5, 15, and 25°C compared to the bog samples, which we attributed to differences in soil properties and initial microbial biomass. The bog sample with added N produced more CO₂ compared to its control, while the fen sample with added P produced more CO₂ compared to its control. It was unexpected that the addition of both N and P reduced CO₂ but increased CH₄ production in both soils compared to their controls. After a month, the pore water C, N, and P stochiometric ratios approached the initial soil microbial biomass ratios, suggesting microbial nutrient recycling in an inherently nutrient-poor soil environment. These preliminary results imply a complex response of carbon turnover in peatland soils to nutrient enrichment.

1 Introduction

Thawing permafrost and collapsing peatlands pose a threat to the stability of the net ecosystem carbon sequestration in subarctic regions (Treat et al., 2019; Schuur et al., 2022). While the extent of permafrost thaw and peatland collapse is rapidly expanding across the region (Porter et al., 2019; Quinton et al., 2019; Hugelius et al., 2020), wildfires have also been increasing

in western Canada (Gibson et al., 2018), Siberia (Talucci et al., 2022), and Alaska (Mekonnen et al., 2022). Increases in the number and extent of fire events result not only in the immediate carbon loss through biomass burning but also an increase of downstream export of particulate and dissolved organic carbon (DOC) from the burned areas as summarized in Table 1 (Burd et al., 2018; Burd et al., 2020; Ackley et al., 2021; Koch et al., 2022; Mekonnen et al., 2022).

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Increasing nutrient inputs to peatland ecosystems is a rising concern for the large storage of carbon in peat soils. Recent observations in western Canada reported that the surface water and shallow groundwater in burned areas were substantially enriched in dissolved nitrogen (N) and phosphorus (P), and even the high P concentrations persisted for several years after a

40 fire (Emelko et al., 2016; Van Beest et al., 2019; Emmerton et al., 2020; Orlova et al., 2020). Thawing permafrost releases dissolved nutrients previously bound in frozen sediments (Treat et al., 2019; Schuur et al., 2022; Wright et al., 2022), while at a landscape level, thaw-induced ground subsidence increases the hydrological and geochemical connectivity among landscape components (Connon et al., 2014; Gibson et al., 2018; Haynes et al., 2018; Post et al., 2019; Carpino et al., 2021) such as forests, fens, thermokarst bogs and other peatlands.

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Table 1: Nitrogen (N) and phosphorus (P) concentrations in wildfire-impacted water samples from recent studies in western boreal and subarctic Canada.

Site	Disturbance	Sampling and analysis methods	N reported (mg L^{-1})	P reported (mg L^{-1})	Reference
Scotty Creek and Notawohka Creek catchments, NWT	Wildfire 3 years ago (the 2013 Notawohka fire)	Burned catchment downstream water sampling	0.5-0.9	0.06	Burd et al. (2018)
Scotty Creek and Notawohka Creek catchments, NWT	Wildfire 3 years ago (the 2013 Notawohka fire)	Burned peatland porewater field sampling and light exposal incubations	1.4-2.8	0.6	Burd et al. (2020)
Fort McMurray, Alberta	Wildfire in the same year (the May 2016 Fort McMurray wildfire)	Surface water samples collected from high- to low-order rivers	2-3	0.5-1	Emmerton et al. (2020)
Pelican Mountain, Alberta	Prescribed burn at research site in the same year	Shallow groundwater samples from monitoring wells	1-3	0.7	Orlova et al. (2020)
Scotty Creek watershed, NWT	Wildfire 2 years ago (2014 low-severity fire in the headwater areas)	Burned peatland porewater, field collected; comparison with unburned area	1-7	0.5-6	Ackley et al. (2021)
Pelican Mountain, Alberta	Laboratory simulated burning of research site samples at 250 and 300°C	5 g of burned peat leached by 1 litre water for 2 days	220-420	12-26	Wu et al. (2022)

Peatlands are generally poor-nutrient ecosystems with respect to both plants and soil microbial communities, while the

50 magnitude of nutrient limitation varies with landscape position, peatland type, and groundwater connectivity (Hill et al., 2014; Lin et al., 2014; Moore et al., 2019). For example, ombrotrophic isolated bogs rely on the atmospheric deposition of N and P and N fixation by N-fixing microbes, generally most poor in soil nutrients. Minerotrophic channel fens receive more nutrients

from surrounding water bodies through groundwater and surface water connections. Although the impacts of changing nutrient levels in temperate and tropical peatland soils have been studied in relation to the agricultural expansion and fossil fuel-driven atmospheric deposition (Amador and Jones, 1993; Qualls and Richardson, 2000; Lin et al., 2014; Hoyos-Santillan et al., 2018; Moore et al., 2019; Schillereff et al., 2021), the results are considered not directly applicable for the remote subarctic peatlands.

The Scotty Creek watershed, Northwest Territories, Canada, hosts one of the main research stations in the subarctic region, with active monitoring and field investigations on rapidly changing discontinuous permafrost landscapes and potential biogeochemical climate feedbacks (http://www.scottycreek.com/). Recent observations pointed to the increase of wildfire impact, the acceleration of permafrost thaw and the degradation of permafrost stability, with peatland ecosystems and their associated hydrological processes and pathways rapidly transforming (Gibson et al., 2018; Ackley et al., 2021; Wright et al., 2022), but the effects of sudden nutrient inputs to the peatland soils remain poorly understood. In this study, our aim was to investigate whether the addition of dissolved inorganic N and P facilitates organic matter decomposition and nutrient recycling in subarctic peatland soils. Also, we anticipated that variations in initial nutrient ratios (N:P, C:N, C:P) and the existing adaptations of soil microbial communities would contribute to understanding the short-term responses of different types of peatlands to additional N or P (Hill et al., 2014; Hoyos-Santillan et al., 2018). A laboratory soil incubation experiment was conducted to compare the CO_2 and CH_4 production rates in the field-sampled bog or fen soils under various treatments: control, N only, P only, and both N and P (NP hereafter) addition. The main incubation lasted for 30 days , subject to fluctuating temperatures, to assess the temperature sensitivity of underlying biogeochemical processes.

2 Methods

2.1 Scotty Creek field sites and peat coring

The sampling locations were within the Scotty Creek drainage basin, Northwest Territories, Canada, which lies within the Taiga Plains Ecozone (Fig. 1). Approximately 25% of this ecozone is covered by wetlands (Mahdianpari et al., 2021) with an estimate of 71,600 km^2 bog and 5,100 km^2 fen peatlands (Webster et al., 2018). The Scotty Creek basin is underlain by discontinuous permafrost that supports a peatland complex with peat plateau forests, thermokarst bogs, channel fens, and open water ponds. In October 2020, two duplicate shallow peat cores (0-25 cm) were taken from each of two sites: a thermokarst bog (hereafter referred to as 'bog peat' or 'bog soil') and a channel fen (hereafter referred to as 'fen peat' or 'fen soil'). These cores were then transported to the University of Waterloo, Waterloo, Canada, and stored in a -20°C freezer until being thawed to start the experiment.
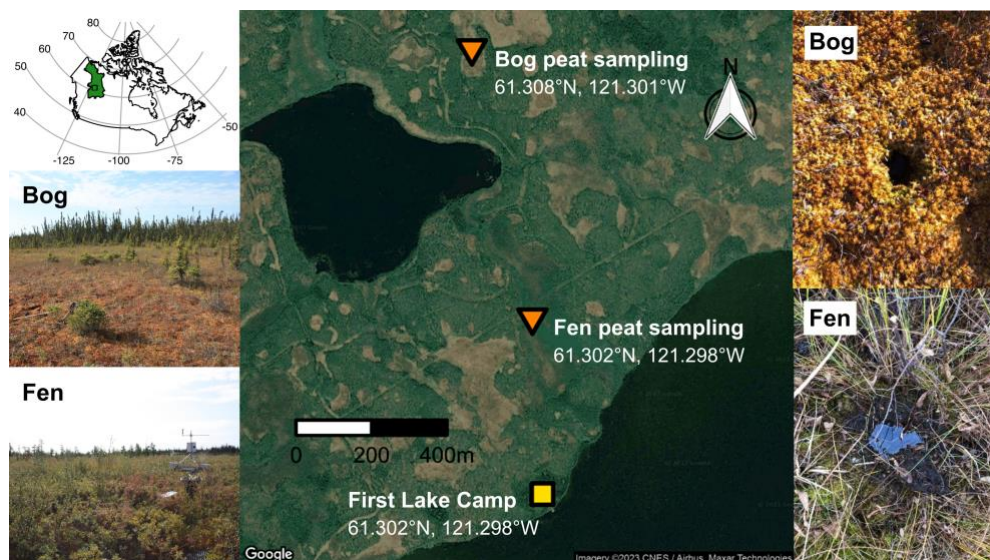
Figure 1: Scotty Creek watershed bog and fen peat sampling site locations (Map data: Google ©2023 CNES / Airbus, Maxar Technologies) and photos (credit: Mason Dominico). The location and extent of Taiga Plains Ecozone of Canada (National Ecological Framework for Canada, 2017) is shown in the inset map with a small box indicator for the site locations. Please replace "First Lake Camp" with "Scotty Creek Research Station". I would also label the larger lake "Goose Lake" because it appears as such on most maps.

2.2 Sample preparation and pre-incubation

The frozen peat cores were thawed at room temperature (22˚C). During the thawing, each peat core was sealed inside its original liner (from the field sampling) to preserve initial porewater release upon its thawing. After 24 hours, the liners were opened carefully, the released porewater from each core was collected and aliquots were saved for chemical analyses. The rest of the wet peat was placed in a clean plastic container and gently hand mixed to take small aliquots for determining approximate values of initial peat bulk density (approximate as the peat was de-structured), and moisture content (by oven-drying at 80˚C for 48 hours). In addition, some of the peat was freeze-dried for total organic carbon (TOC) and nitrogen (TN) contents using a CHNS analyzer (Carlo Erba NA-1500 Elemental Analyzer; detection limit of 1% by mass for each parameter) and for total phosphorus content by magnesium nitrate ($MgNO_3$) digestion, ashing in a muffle furnace at 550˚C, and hydrochloric acid extraction (Aspila et al., 1976). However, we did not attempt to interpret the total mass balance results for total pools due to the addition of extra salts and mass for nutrient treatment.

The containers with bulk peat samples were placed inside an environmental chamber at a constant temperature of 5˚C for two weeks. During this pre-incubation, the peat containers were uncovered inside the chamber, allowing for aerobic conditions. The moisture contents of peat were reduced but not desiccated (i.e., peat was visually moist, but no water comes out when it was gently squeezed). The moisture contents by oven-drying were repeated after this pre-incubation period, to decide how much porewater solutions to add to return the peat to the field conditions.

After the pre-incubation, each of the bog and fen peat samples was subsampled for fumigation to measure microbial biomass C, N and P concentrations. Then, the rest of the bulk peat was allocated into 250 mL mason glass jars (bog: 55 g peat and fen: 40 g peat). Porewater base solutions were prepared by diluting the initial thaw-collected porewater around 10:1, to make the required volume of the base solution while accounting for pH and initial nutrient levels, rather than using pure water. The total amount of the porewater base solutions needed for the incubation jars was estimated based on the desired peat moisture levels during the incubation (volumetric water content of 80% for bog peat and 100% for fen peat as approximate growing-season averages measured in adjacent meteorological stations).

The artificial porewater solutions were prepared, to adjust the peat moisture content and different treatments, by adding different amounts of salts including monobasic sodium phosphate (NaH_2PO_4), ammonium nitrate (NH_4NO_3), and ammonium chloride (NH_4Cl) for the N and/or P porewater enrichment scenarios. This experiment was aimed to test high levels of porewater nitrogen (7 mg-N L^{-1}) and phosphorus (6 mg-P L-1) from previous study measurements (Table 1). For the N amendment, the mix of NH_4NO_3 and NH_4Cl was used to introduce a 3:1 ratio for NH_4-N to NO_3-N following the measurement from a burned peat surface (Table 1). The solutions were added to bog and fen peat samples for control, N added, P added, and both N and P added, all prepared in duplicate (4 treatments × 2 sites × 2 samples = 16 total incubation jars). After adding the solutions, the headspace was 100 mL for bog samples and 150 mL for fen samples. The jars were placed back in the environmental chamber at 5°C and pre-incubated for four weeks until stable CO_2 efflux rates were measured.

2.3. Incubation experiment and subsampling

The incubation jars were left open inside the environmental chamber to maintain aerobic headspace conditions, except during the closed headspace gas sampling. To maintain the moisture contents in the peat samples, the jars were placed together in a partially opened plastic bag with a small beaker containing a Milli-Q water-soaked sponge. The potential evaporative water loss was monitored by measuring the weights of two additional jars containing the same quantity of bog and fen peat, going through the same temperature course. Using this information, the peat moisture content was maintained within 10% of the initial condition by spraying some Milli-Q water inside the plastic bags to compensate for evaporation loss throughout the incubation experiment.

During the one-month incubation experiment, the headspace gas sampling was performed every third day once the chamber was set to a new temperature. Stepwise changes in the chamber temperature from 1°C, 5°C, 15°C, 25°C, 15°C, 25°C, 15°C, 5°C, 1°C, 5°C and back to 1°C were imposed every 3-day interval to roughly mimic field situations where the ground temperature fluctuates during the non-growing to growing season transition. This non-growing to growing season transition, which includes snow melt, was thought to be a plausible scenario for the sudden contact of nutrient enriched water for distant

peatlands after a watershed fire event. The comparison of flux rates at various temperatures was also aimed to test potential variation in nutrient interaction with microbial activities.

140 **2.3.1 Porewater chemistry**

Approximately 20 mL of porewater was extracted from bog and fen peat samples during the pre-incubation phase, and from each incubation jar during the post-incubation phase. The chemical properties of the porewater collected from the initial thawing of the peat cores were used for determining the initial porewater conditions and preparation of the artificial porewater solutions. The post-incubation porewater was collected by gently squeezing the saturated peat inside each incubation jar. The

145 porewater pH was measured by a calibrated electrode (Orion™ Economy Series pH Combination Electrode, ThermoScientific) before filtering. The rest of the porewater samples were filtered through a 0.45 µm pore size membrane filter (nylon membrane syringe filters, VWR Scientific) and stored at 4°C in the refrigerator if not analyzed immediately.

Concentrations of dissolved organic carbon (DOC), total dissolved nitrogen (TDN), and dissolved inorganic carbon (DIC) in

150 the filtered porewater were measured using a total organic carbon analyzer (TOC-LCPH/CPN, Shimadzu; method detection limit 3 µmol L^{-1}). For the measurement, 1 mL of porewater was filtered through a 0.2 µm pore size membrane filter (Polyethersulfone membrane syringe filters, Thermo Scientific) and frozen for subsequent analysis of major anion concentrations including chloride (Cl^-), nitrite (NO_2^-), nitrate (NO_3^-), and sulfate (SO_4^{2-}) using ion chromatography (IC, Dionex ICS-5000 with a capillary IonPac® AS18 column; ± 3.0% error and ± 1.6% precision; method detection limit 0.59, 1.29, 1.13,

155 1.47 µmol L-1, respectively). Porewater ammonium (NH_4^+) and dissolved reactive P (DRP) concentrations were measured spectrophotometrically on a Thermo Scientific™ Gallery™ Discrete Analyzer (±10% error and ±3% precision). The concentrations of major cations including dissolved calcium, iron, potassium, magnesium, manganese, sodium, sulfur, and silicon concentrations were measured using Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES, Thermo Scientific iCAP 6300 Duo).

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2.3.2. Microbial biomass fumigation

The microbial biomass carbon (MBC), nitrogen (MBN) and phosphorus (MBP) concentrations of the peat samples were measured using the chloroform fumigation method (Brookes et al., 1984; Vance et al., 1987; Joergensen, 1996; Jenkinson et al., 2004). Approximately 4 g of peat was used for OC and N extraction with 0.5 M potassium sulfate, or for P extraction with

165 0.5 M sodium bicarbonate (NaHCO₃ solution with pH adjusted to 8.5). The subsamples were treated either with chloroform for 24 hours to fumigate in a vacuum desiccator, or with no fumigation. The potassium sulfate extract was filtered through a 0.45 µm pore size membrane filter (nylon membrane syringe filters, VWR Scientific), and the extracts were measured for DOC and TDN. The sodium bicarbonate extract was also filtered through the 0.45 µm filter, and the extracts were measured

for total dissolved phosphorus (TDP) using ICP-OES. The difference in DOC, TDN, and TDP concentrations between the
fumigated and non-fumigated samples represented the C, N, and P present in the microbial biomass. An extraction efficiency
of 0.45 was assumed for MBC and 0.54 for MBN (Joergensen, 1996), and 0.4 for MBP (Jenkinson et al., 2004).

2.3.3. Headspace CH_4 and CO_2 flux measurements

The changes in CO_2 and CH_4 concentrations in the headspace of the jars during the incubation were measured by closing the
jars for 15 or 20 minutes. Up to 10 mL headspace gas samples were taken from each jar at the end of the closed incubation
through the custom lid with three-way gastight valves and a 10- or 20-mL plastic syringe. The CO_2 and CH_4 concentrations
were analyzed by direct sample injection into a Gas Chromatograph (Shimadzu, Model GC-2014) equipped with a flame
ionization detector and methanizer. The method detection limits for CH_4 and CO_2 were 0.384 and 17.145 ppm, respectively.
For gas samples with CO_2 concentrations greater than 1,000 ppm, the samples were diluted with 15-30 mL of helium gas and
allowed to mix for 20 minutes before analysis on the Gas Chromatograph. Gas efflux rates (F_{gas}, μmol g-1 h-1) were calculated
following Eq. (1):

$$F_{gas} = \frac{PV_H(C_{t1}-C_{t0})}{RTm\Delta t} \tag{1}$$

where P is the headspace pressure (atm), V_H is the headspace volume (L), R is the gas constant (0.0821 L atm mol^{-1} K^{-1}) and T
is the temperature (K). Gas efflux was estimated using the relative headspace concentration changes inside the closed jar
environments, as $C_{t1}-C_{t0}$ (ppm or 10-6) for each gas species during the time, $\Delta t=t_1-t_0$ (h). The gas efflux rates were then
normalized per dry mass of peat (m, g dry peat) determined at the beginning of the incubation experiment (the mass loss was
assumed minor and not considered in the flux rate calculations).

3 Results and Discussion

3.1 Transient changes in the soil CO_2 and CH_4 production

The fen soil incubation resulted higher CO_2 and CH_4 production rates than the bog soil incubation as the incubation temperature
was cycling from 1 to 25°C (Fig. 2). The CO_2 production rates of the bog samples were about 50% of those of the fen samples
and the CH_4 production rates of the bog samples were an order of magnitude smaller than those of the fen samples. The higher
CO_2 and CH_4 production rates in the fen soil are consistent with its higher microbial biomass (MBC 4.3 mg C g^{-1}) than the bog
(MBC 0.7 mg C g^{-1}) initially.

The temperature effects complicated the interpretation of the resulting gas production rates. The bog soil with added P and NP
resulted distinctively lower CO_2 production rates at 25°C compared to the other treatments, while the same P additions resulted
higher CO_2 production rates at 15°C compared to the N only addition (Fig. 2a). The fen peat with added N and NP resulted

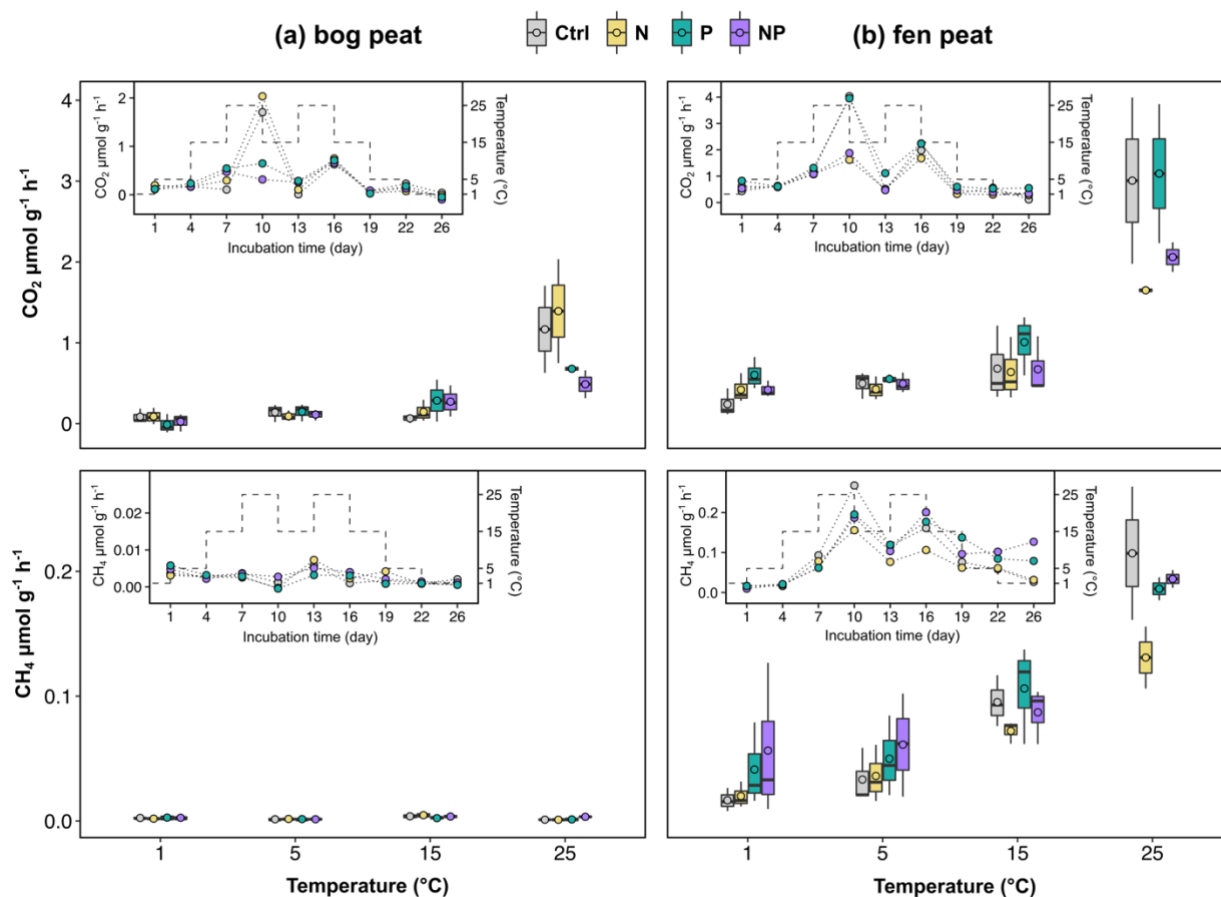
lower CO_2 production rates at 25°C, while the same N additions showed no apparent divergence at 15°C compared to the
200   control, but instead the P only addition having distinctively higher rates than the other three (Fig. 2b). The CH_4 production
rates of the fen soil also presented noticeable differences by temperature as well as nutrient (Fig. 2b) without a clear trend. The
complex responses of soil carbon gas production may reflect inherently different limiting nutrient in either type of peatland
(more discussed in 3.3).

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Figure 2: The CO_2 and CH_4 flux rates from the laboratory incubation measurements for subarctic (a) bog and (b) fen peat samples.
Inset figures show the rate changes (connected dots) for each nutrient treatment (Ctrl: control; N: nitrogen addition; P: phosphorus
addition; NP: nitrogen and phosphorus addition) over the incubation period with temperatures shown as dashed step lines. Box
plots show the summary of rates at each temperature (median line and mean circle in each box) for each treatment.

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3.2 Variations in the temperature sensitivity of CO_2 and CH_4 productions

In a broad sense, the CO_2 and CH_4 production rates followed the anticipated exponential increase with temperature (Fig. 2). Based on the average gas production rates at 5°C and 25°C, the temperature sensitivity parameter Q_{10} was calculated using an exponential rate increase following Eq. (2)

215 $$Q_{10} = (\frac{R2}{R1})^{(\frac{10}{T2-T1})} \tag{2}$$

where $R2$ is the rate at the incubation temperature 25°C ($T2$) and $R1$ at the 5°C ($T1$) (Table 2). The Q_{10} values shows that the additional nutrients reduced the temperature sensitivity of the CO_2 emissions (i.e., the Q_{10} values were lower), except for the only N addition to the bog soil. According to the flux rate summary in Fig. 2, the low Q_{10} values correspond to relatively slow flux rate increases with temperature, while the higher Q_{10} represents the relatively fast flux rate increase with temperature rise

220 to 25°C. For example, the NP addition resulted the lowest CO_2 production rates in the bog soil at 25°C, contributing to the apparently lowest Q_{10} of 2.09 (Table 2); in contrast, the N only addition resulted the highest CO_2 production rate in the bog soil at 25°C, contributing to the highest Q_{10} of 3.89 (Table 2).

Table 2: Estimated temperature sensitivity based on the average gas production rates at 5°C and 25°C.

Treatments	Bog CO_2	Fen CO_2	Fen CH_4
Control	2.89	2.46	2.54
N addition	3.90	1.97	1.91
P addition	2.15	2.37	1.93
N P addition	2.09	2.04	1.78

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The estimated temperature sensitivity here suggests relatively high CO_2 production rates at low temperature from alleviated nutrient limitations (as in low Q_{10} values of both NP, bog P, and fen N), high CO_2 production rates at high temperature by effective mitigation of lacking nutrients (as in high Q_{10} of bog N and fen P), or both trends combined. Recently, Liu et al. (2022) suggested that the catalytic efficiency of hydrolytic enzymes to facilitate soil N and P recycling largely increased with

230 temperature, which supports the second suggestion of effective mitigation of lacking nutrients (i.e., soil respiration at warmer temperature becomes less dependent on the initial nutrient shortage). Determining Q_{10} parameter for varying peatland classes has been an important task for process-based C cycle models (Bona et al., 2020), and the recently found latitudinal increase of temperature sensitivity (Byun et al., 2021) might actually reflect the increasing severity of nutrient limitation towards high latitude peatlands.

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3.3 Cumulative carbon release by nutrient addition

The stoichiometric ratios of primarily accessible substrates are important for soil microbial C cycling. The nutrient element ratios in the soil solution relative to the microbial biomass ratios can determine the use efficiency of DOC for respiration or biomass storage. This process has been conceptualized as microbial carbon use efficiency (CUE), which is expressed by the

240   fraction of substrate OC that is taken up into microbial biomass of the total carbon that is used for both biomass uptake and cell metabolism (Manzoni et al., 2012; Sinsabaugh et al., 2013; Sinsabaugh et al., 2016; Geyer et al., 2016). If microbial cells are substantially limited by available nutrient N and/or P, they may allocate less of the DOC for biomass growth but still respire more (producing more CO_2 from DOC) for alleviating the nutrient limitation (thus, low CUE). To discuss this effect, the comparison chart for the cumulative emission of CO_2, CH_4 and the total of both gases (Gas C) is shown in Fig. 3, as the

245   cumulative emissions were considered more indicative of the resulting net changes by the initial nutrient additions in peat carbon cycling over incubation.
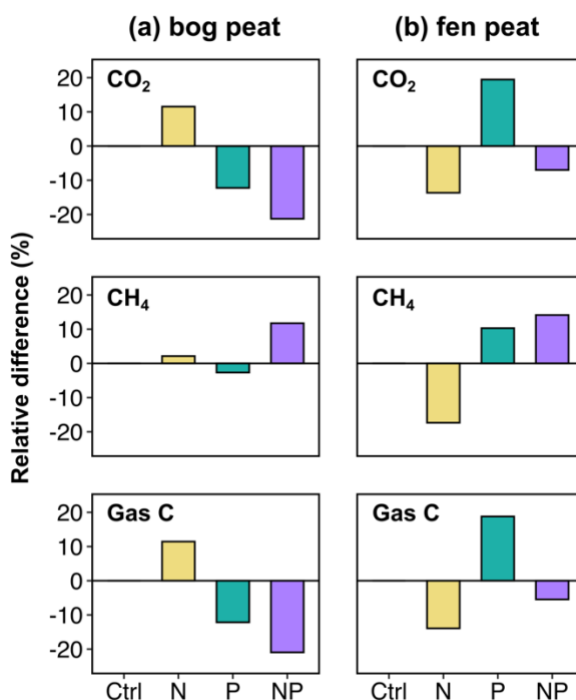
Figure 3: Relative differences (%) in the cumulative CO2, CH4, and total gas carbon (Gas C) emissions observed throughout the
250   **incubation.**

The microbial CUE was estimated for the bog soil by fumigation extraction method at the end of the incubation (Table 3). The fen soil fumigation experiment was not successful yielding negative microbial biomass calculations. Then, the microbial CUE of bog soil was approximated by the gross MBC acquisition, divided by the total of the MBC acquisition and the cumulative

255 gas C release. As a result, the lower CUE by the N addition was attributed to the largest C gas release with the smallest gross MBC acquisition. The highest CUE value with the larger MBC acquisition and the smaller cumulative C gas release was found in the case of NP addition. This partially corresponds to the above interpretation of temperature sensitivity trend that the microbial respiration activities increased to alleviate relatively lacking nutrient P in the bog soil in response to the imbalance intensified by N addition.

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Table 3: Microbial biomass changes and carbon use efficiency (CUE) of bog peat.

Extraction	Before			After				
Bog	MBC (mg C g^{-1} dry soil)	MBN (mg N g^{-1} dry soil)	MBC:MBN (atomic ratio)	MBC (mg C g^{-1} dry soil)	MBN (mg N g^{-1} dry soil)	MBC:MBN (atomic ratio)	Gas C (mg C g^{-1} dry soil)	Approx. CUE
Control	0.69	0.10	8.1	8.5	0.97	10.2	4.0	0.49
N addition	0.69	0.10	8.1	6.8	0.79	10.0	4.5	0.26
P addition	0.69	0.10	8.1	7.7	0.86	10.4	3.5	0.50
NP addition	0.69	0.10	8.1	9.8	1.2	9.7	3.2	0.65

The mere amount of OC substrates is likely not at all limiting in peatland soils, and microbial strategies to overcome relative nutrient imbalances may fall into three broad categories: (1) increase the production of extracellular enzymes to acquire
265 necessary nutrient elements from enzyme-facilitated organic matter degradation, (2) recycle the nutrient elements assimilated in dead microbial cells (i.e., necromass recycling), and (3) release extra substrate C via respiration without biomass assimilation (i.e., overflow respiration) (Giesler et al., 2011; Manzoni et al., 2012; Lin et al., 2014; Hoyos-Santillan et al., 2018; Schillereff et al., 2021; Lu et al., 2022). The quality or degradability of C substrates may have a determining effect on which strategy to be activated by soil microbes, which in turn accelerates or decelerates the overall decomposition rate of soil organic matter
270 (SOM).

3.4 Stoichiometric changes in soil solution

Given the dark chamber environment used for the incubation and no additional organic substrate, the changes in the porewater pools were considered fully the heterotrophic metabolism of existing pools. The changes in the DOC concentrations with
275 stoichiometric ratios before and after the incubation are shown in Fig. 4. The initial microbial biomass stoichiometry is added as a dotted line in each panel of Fig. 4. Overall, the DOC decreased from the initial concentrations, but the remnants were more than the estimated consumption from the cumulative carbon gas emissions, indicating the use of solid organic particles. The final concentration of DOC in the bog with NP addition (78.2 ± 6.88 mg C L^{-1}, mean ± SD of duplicates) was smaller than the control (101.6 ± 9.97 mg C L^{-1}), while the N-only or P-only addition was not meaningfully smaller than the control (97.0

280 ± 0.69 mg C L-1 for the N addition; 94.3 ± 1.19 mg C L-1 for the P addition) (Fig. 4a). In the fen, the final DOC concentrations were all around the control result (Fig. 4b).

The TDN by the end of the incubation was around approximately 40-60% of NH_4^+ in both peat types (e.g., NH_4^+ 2.3-3.8 mg-N L-1 and TDN from 5.2-6.1 mg-N L-1 in bog control to N added; NH_4^+ 5.2-7.7 mg-N L-1 and TDN 13.3-15 mg-N L-1 in fen

285 control to N added). In theory, the NH_4^+ would have been taken up by plants under field conditions. The majority of the remaining TDN was dissolved organic N with minor contributions by NO_3^- or NO_2^- to the TDN. These porewater patterns may provide some explanation for the reported water quality changes in burned catchments, lasting high phosphate and high dissolved organic N concentrations (Ackley et al., 2021; Burd et al., 2020; Wright et al., 2022).

290 The stoichiometric ratios of DOC to TDN and TDP in the porewater approached the initial microbial biomass ratios by the end of the incubation of both bog and fen peat. In the bog peat, as the relative N shortage by the initial P addition reduced both MBC growth and cumulative respiration, the CUE was estimated close to the control while the N addition resulted in a much smaller CUE (Table 3). This could be due to the microbial community of bog needing relatively less energy for the necessary N acquisition than for the P acquisition from the peat environment. The bog peat microbial community may have specifically

295 adapted to the constant shortage of available P in peat, allowing them to release as much bioavailable P as required to overcome the nutrient imbalance for C cycling, even with a high energy cost (Hoyos-Santillan et al., 2018). In the fen peat, P release seemed to be an efficient process for the fen adapted microbial community, given the prompt enzymatic DRP release to meet the increased need by the N addition.

300 In the bog peat, the largest net microbial biomass growth occurred when NP were added, possibly by more direct assimilation of available DOC instead of auxiliary respiration (Giesler et al., 2011; Manzoni et al., 2012; Sinsabaugh et al., 2013; Lin et al., 2014). It seems that the NP addition could best enhance the microbial CUE while reducing the auxiliary CO_2 production, while also more quickly consuming available DOC and further degradation of solid carbon, which under the anaerobic soil condition resulted the higher cumulative CH_4 production (Fig.3).

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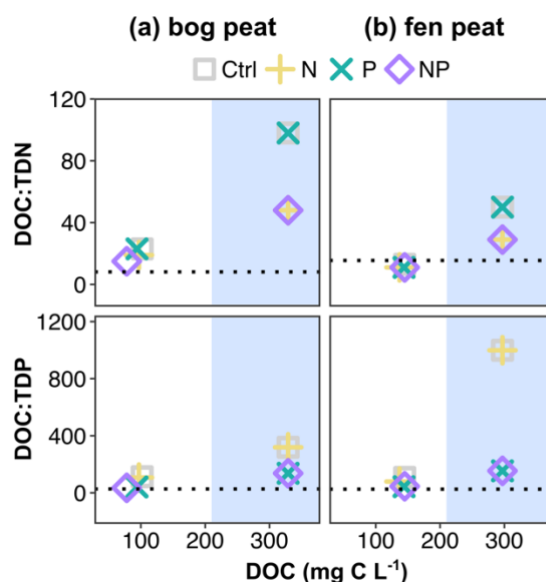
Figure 4: Porewater dissolved organic carbon and stoichiometric ratio change before and after the incubation. The shaded area indicates where the initial porewater ratios are marked. The dotted lines represent the initial microbial biomass atomic ratios (bog peat MBC/MBN = 8.1, MBC/MBP = 26.9; fen MBC/MBN = 15.5, MBC/MBP = 25.6).

310

Despite the aerobic headspace setting of incubation jars, the water saturated peat environments initiated anaerobic microbial metabolism, as indicated by the net CH_4 production and release (Fig. 2). The increase of porewater dissolved iron (Fe) and manganese (Mn) is also indicative of the reductive dissolution of sediment iron and manganese oxides (Table 4). As the DRP concentrations increased in the fen samples, we interpret that some of this DRP production may have been associated with the

315 release of DRP that was bound to Fe and Mn oxides (Table 4). To attribute the net DRP production to Fe and Mn oxide reduction versus biological sources of DRP production such as enzymatic release, we subtracted the amount of DRP release that was possible from Fe and Mn oxide reduction using the maximum molar ratio of 1 mol Fe and Mn to 1 mol DRP (Orihel et al., 2017). As a result, the largest biological DRP release was measured in the N addition and the smallest amount was released in the NP addition. In the bog peat, net DRP uptake was estimated (Table 4), implying the inherent limitation of

320 available P for microbial communities.

Table 4: Porewater phosphorus changes from the initial to the final analysis.

Porewater		Initial		Final		Final – Initial				Approx. DRP
		DRP (μmol L^{-1})	TDP (μmol L^{-1})	DRP (μmol L^{-1})	TDP (μmol L^{-1})	ΔDRP (μmol L^{-1})	ΔTDP (μmol L^{-1})	ΔFe (μmol L^{-1})	ΔMn (μmol L^{-1})	production[a] (μmol L^{-1})
Bog	Ctrl	67.9	86.0	64.8	75.2	-3.1	-10.8	1.05	0.43	Net uptake
	N	67.9	86.0	56.9	75.6	-11.0	-10.4	0.89	0.41	Net uptake
	P	182	200	146	193	-36.0	-7.0	0.79	0.46	Net uptake
	NP	182	200	160	187	-22.0	-13.0	0.80	0.44	Net uptake
Fen	Ctrl	22.9	24.8	98.2	112	75.3	87.2	15.5	6.54	53.3

	N	22.9	24.8	135	146	112	121.2	12.6	6.85	92.7
	P	159	161	217	282	58.0	121.0	13.8	6.11	38.1
	NP	159	161	205	261	46.0	100.0	14.0	5.33	26.7

[a] Hypothetical DRP production that could be attributed to enzymatic release; for the bog porewater measurements, net DRP uptake was estimated, and thus the DRP production was not determined.

325

These observations support the critical role of microbial metabolism in regulating nutrient forms and concentrations in peat porewater and imply the role of peatlands in large scale on the watershed nutrient fluxes and water quality (Liu et al., 2023). Relying on the internal supply of C substrates from peat decomposition, the fen porewater ended up accumulating additional dissolved inorganic P, possibly in the process of balancing for the bioavailable substrate stoichiometry by microbial activities.

330 In contrast, the net uptake of P by the bog peat in all four of the treatments suggests that bog peatlands might have some capacity to sequester P inputs via microbial assimilation because P is the inherently limiting nutrient for microbial growth (Worrall et al., 2016). However, the stability and time scale of this P sequestration into microbial biomass in the bog soil remains uncertain.

4 Future research suggestions

335 The experiment in this study tested the instantaneous responses of peat microbial community to a one-time, or pulsed, nutrient nitrogen and phosphorus addition. In a real-world situation, multiple possible scenarios could occur in terms of the variability in the concentrations, ratios, timing, and duration of such nutrients that may be delivered to peatlands, while the combined effects of permafrost thaws and frequent wildfires are likely to increase dissolved nutrients in subarctic peatlands, previously considered remote and less affected by industrial inputs. Future experiments should more systematically investigate various

340 nutrient supply scenarios across different time scales and peatland types. Understanding the microbial regulation of porewater stoichiometry in response to increased nitrogen or phosphorus availability may be crucial in explaining sudden changes in CO_2 and CH_4 emissions from peatlands. We recommend future work to explore the existing inherent nutrient limitation of the peatland microbial communities and their response to both pulsed and continuous nutrient inputs with a wide range of representative seasonal temperature fluctuations. Consequently, peatland carbon cycle models should also account for the

345 alteration of the temperature sensitivity parameter by shifts in nutrient concentrations and ratios.

Data availability

The data that support the findings of this study are available at https://doi.org/10.20383/102.0712 in Federated Research Data Repository (FRDR).

Author contribution

350 **Eunji Byun**: Conceptualization, Methodology, Investigation, Writing - Original draft preparation. **Fereidoun Rezanezhad**: Conceptualization, Supervision, Writing - Review & Editing. **Stephanie Slowinski**: Conceptualization, Methodology, Validation, Writing - Review & Editing. **Christina Lam**: Methodology, Writing - Review & Editing. **Saraswati Saraswati**: Validation, Writing - Review & Editing, **Stephanie Wright**: Resources, Validation, Writing - Review & Editing. **William L. Quinton**: Resources, Validation, Writing - Review & Editing. **Kara Webster**: Writing - Review & Editing. **Philippe Van**
355 **Cappellen**: Conceptualization, Supervision, Writing - Review & Editing.

Competing interests

The authors declare that they have no conflict of interest.

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